THE EFFECTS OF ALUMINUM AND ACIDITY ON THE

DRIFT, MORTALITY, AND MOLTING OF STREAM

INSECTS

William Cook
and
James Haney

THE UNIVERSITY OF NEW HAMPSHIRE

WATER RESOURCE RESEARCH CENTER

Durham, NH 03824

ABSTRACT

Responses of nine stream insects to short term increases of acidity and aluminum concentrations in artificial streams were studied. For the mayflies studied (Stenonema modestum Ephemerella subvaria and E. catawba) drifting behavior increased when aluminum concentrations were elevated above 1 ppm and mortality was increased at pH<5. Three of the four caddisflies studied (Hydropsyche spp., Macrostemum zebratum, and Chimarra obscurra) were affected by increased salinities in high aluminum treatments, but were not adversely affected by short term increases in acidity or aluminum concentrations. The fourth caddisfly studied (Potamyia flava) suffered increased mortality at pH<5, and when aluminum concentrations rose above 1 ppm. The stonefly (Amphinemura nigritta) had high total population losses at high aluminum concentrations and at pH<5. The water penny beetle (Psephenus herricki) showed no response to short term water chemistry alterations, but long term exposure to reduced pH resulted in high drift losses of this insect. Molting success of the insects was reduced by increased acidity.

INTRODUCTION

Changes in precipitation chemistry caused by industrial pollutants have been acknowledged for many years (Likens and Bormann, 1974). Recently the influence of "acid precipitation" on leaching of cations from soil into streams has been investigated (Johnson et al., 1981; Webb, 1982). During short periods of warm weather in spring, melting of acidified snow may cause short term increases in cations, especially potassium and aluminum, that leach into streams (Cronan and Schofield, 1979). This effect is most pronounced when deep thick snow cover reduces ice formation in the soil (Johnson et al., 1981). The objective of this study was to investigate the

effects of these short term alterations of aluminum and acidity upon drift
and mortality of stream insects.

Other investigations on the effects of acidity and heavy metals upon
stream insects have involved alterations of water chemistry under laboratory
conditions to compute an LD_{50} (e.g. Bell and Nebeker, 1964; Bell, 1971) or
altered chemical conditions of natural streams (Koryak et al., 1972; Fiance,
1978; Winner et al., 1980; Hall et al., 1980; Peckarsky and Cook, 1981; Havas
and Hutchinson, 1982; 1983). Our study differs from previous investigations
by its concentration upon the effects of chemical alterations upon insect
behavior, such as drift, in addition to the effects of the chemical changes
upon mortality.

In our study, insects collected from riffle habitats were placed in
artificial streams and subjected to aluminum potassium sulfate, sulfuric
acid, and potassium hydroxide. Three effects of these chemicals were then
examined: drift, the movement of live insects out of a habitat; mortality,
the acute physiological reaction to the chemicals; molt failure, inability to
shed the cuticle. Drift, molt success and mortality under varying chemical
conditions were computed for: the caddisflies Macrostemum spp.
(Hydropsychidae), Hydropsyche spp. (Hydropsychidae), Potamyia flava
(Hydropsychidae), and Chimarra obscurra (Philopotamiidae); the water penny
beetle, Psephenus herricki (Psephenidae); the stonefly Amphinemura nigritta
(Nemouridae); the mayflies Ephemerella catawba, E. subvaria (Ephemerellidae)
and Stenonema spp. (Heptageniidae).

METHODS

The insects used in this study were collected from two third-order
rivers in southeastern New Hampshire (Table 1). Three sites on the Oyster
River (Strafford Co. NH) supplied insects: site 1 was in the headwaters of

3

the river; site 2 was above the Durham water supply reservoir; site 3 was below the Durham water supply reservoir. Eutrophication and sediment load increased in the lower elevation sites. The two sites on the North River (Rockingham Co. NH) were adjacent, but had different substrates; site 2 was more sandy and had less macrophytic growth.

For each experimental run the captured insects were sorted into eight groups: Stenonema spp; Ephemerella spp.; Macrostemum; other Hydropsychidae; Chimarra spp.; Brachyptera spp.; Amphinemura spp.; and P. herricki. Approximately equal numbers of each group were then placed into four artificial stream troughs. Density of animals in the troughs were lower than those observed in the field (Tables 1 and 2). The stream troughs were 2.4 m long X 0.15 m wide X 0.15 m high. Brushed and autoclaved stones (less than 10 cm diameter) were used as a substrate. Aufwuchs on the sides of the trough and (later in the experiment) on the stones' surface was available as a grazer food source. Plankton-rich water used in each trough was obtained from the Oyster River below the Durham Reservoir, thus supplying food for the filter-feeding Trichoptera. At the end of each trough water flowed through a net and into a reservoir. The 26 1 of water in each trough was recirculated from the reservoir to the top of the trough by a centrifugal pump at the rate of 24 liters $(min)^{-1}$, but because baffles in the troughs restricted water flow, flow rate was not constant throughout the length of the troughs. Inclination of the troughs was less than 2°. The troughs were housed in an environmental control room where the water temperature was maintained between $9^{\circ}-12^{\circ}$ C and photoperiod adjusted for season. Light was supplied by a bank of fluorescent lights directly above the troughs. The insects were allowed to acclimate to these conditions for 16-20 hours prior to the experiment.

4

Following acclimation, those insects that had drifted during the acclimation period were returned to the top of the trough and water chemistry was altered in three of the troughs. The fourth trough was the control. Acidity was increased by adding 2 N $H_2(SO_4)$; aluminum was increased by adding $AlK(SO_4)$ solution (200 ppm Al). Since $AlK(SO_4)$ is an acid salt, KOH was added to produce low acidity-high aluminum conditions. Treatment troughs received both $H_2(SO_4)$ and $AlK(SO_4)$. Total aluminum concentrations were determined colorimetrically by the ferron-orthophenanthroline method (Rainwater and Thatcher, 1960). Iron and fluorine ions interfere with this determination. Concentrations of iron, tested concurrently with aluminum, and fluorine, tested by the Durham Water Supply, were consistently low and would produce an error in aluminum determination of less than 1%. Speciation of total aluminum was not determined. Hydrogen ion concentrations were measured with a pH probe (Orion Research models 231 and 407a). Chemical conditions were measured at least three times a day. Due to the interplay of aluminum speciation, hydroxide ions and the acidic $AlK(SO_4)$, it was impossible to maintain constant chemical conditions over the three day experiment. Therefore, insect responses were analysed according to the range of conditions experienced in the trough. The pH ranges were 4-5, 5-6, and 6-7, the aluminum concentrations were 1-2.5 ppm (>1 ppm) and <1 ppm.

Insect drift, molting ability and mortality were measured. Drift was recorded by daily removal of drifted insects from the nets at the bottom of each trough. Individuals found dead in the drift were recorded as mortalities rather than drift. Because an individual need not drift upon death, no attempt was made to record mortality on a daily basis. Exuvia, individuals that had died while molting and individuals that had died before fully pigmented following a molt were also recorded in the drift samples.

After recording drift on the third day, stones were removed and washed to collect remaining insects and exuvia from emerged adults. Individuals were recorded as mortalities if no movement could be evoked from the individual, or, if alive, as unaffected individuals. The artificial stream troughs were then reassembled by haphazardly replacing the substrate and rinsing the troughs for 24-48 hours with Durham tap water. Despite these rinses, aluminum concentrations in controls (0.3-0.5 ppm) were higher than those found in the Oyster River (about 0.1 ppm), probably due to aluminum hydroxides not removed by the rinse water contaminating control water. Acidities in control troughs were the same as those measured in the Oyster River (approximately pH7). Tap water was replaced by fresh Oyster River water 24 hours prior to each experimental run. Insects were identified using the keys of Hitchcock (1974), Brigham et al. (1981) and Ross (1944). Macrostemum individuals are probably M. zebratum (Hagen), however, specific identification is difficult. Hydropsyche betteni (Ross) accounted for approximately 48% of the Hydropsyche tested, H. ellisoma (Ross) accounted for approximately 46%. The similarity of these two species made specific identification of some individuals uncertain, therefore they were pooled at the generic level. It is unlikely that this had any effect upon the results of the analyses because responses by these species were very low. Most of the Stenonema tested were S. modestum (Banks); however, identification of Stenonema less than 5 mm in length (18% of the total) were uncertain.

Ten experimental runs were performed between December-February and April-May. Four responses were computed: cumulative drift (the percentage of the initial population in each trough that drifted following three days exposure); daily drift (the percentage of the population that drifted during a 24 hour period); mortality of the intial population following three days

6

exposure; total population losses (the sum of cumulative drift and mortality); molt success (the ratio of successful molts, as indicated by exuvia, to total molts, indicated by exuvia and mortalities that took place during molting).

Responses observed during five different chemical alterations (pH 6-7/[Al]<1 conditions were not tested) and the control treatments following three days exposure were analysed by the G-test of independence (Sokal and Rohlf 1969). This test detects differences in a set of percentages. If a set of percentages are heterogenous (i. e. at least one of the percentages is different from the others) the test is significant. A significant test does not imply two mutually exclusive sets of percentages, usually in significant tests one finds two homogenous sets of percentages with elements that are common to both. A treatment was judged to adversely affect an insect if the percentage of the individuals responding to a treatment (by drifting, dying, or through reduced molting ability) following three days exposure differed significantly (i. e. was heterogenous at $p < 0.05$) from the percent response in control treatments and the response was greater than that observed in the control treatments. Because the G-test can be employed only on sets of percentages in which no observations are 0, the G-test was only used to detect differences among non-zero percentages. The values of G cited in RESULTS are the values reflecting the probability of heterogeneity among all non-zero percentages. To find differences between treatments and controls the G-test was performed on progressively smaller sets of percentages until two different homogenous sets were found. Unless otherwise stated, all significance levels are at $p < 0.05$.

If an insect was adversely affected by aluminum, it was further tested for any responses to increased salinity. We hypothesized that effects

7

attributed to aluminum may have been due to K^+ or SO_4^- ions also added with the $K_2(SO_4)$. These ions were at especially high concentrations in high aluminum-low acidity treatments when aluminum hydroxide precipitation was high and large amounts of $AlK(SO_4)$ were added to maintain high aluminum concentrations. Potassium concentrations in these treatments were 8×10^{-5} M by the end of the experiment. To test for salinity effects, test insects were treated as outlined above, except that $K_2(SO_4)$ was substituted for $AlK(SO_4)$. Thus, these treatments represent a repetition of high aluminum treatments with potassium ions substituted for the aluminum ions. These salinity treatments were performed at each of the pH ranges. Percent response was compared with that observed in high aluminum treatments by finding the set of responses (control or treatment) that did not differ from the response to salinity.

RESULTS

Ephemeroptera

Stenonema modestum (Banks)

The differences between cumulative drift of Stenonema spp. were dramatic (Fig. 1). Indeed, the cumulative drift in pH 5-6/[Al]>1 ppm and pH 4-5/[Al]>1 ppm treatments (>24%) were so high that these two percentages were significantly different from all other treatments and the control (<11%) (Fig. 1; $G=60.48$, df=5, $p \ll 0.005$). Daily drift rates were also high in moderate-high acidity/high aluminum treatments (Table 3), resulting in average daily drift rates more than three times higher than other treatments. Drift was not due to the high salinities in these treatments. Cumulative drift in high $K_2(SO_4)$ treatments was 4% at pH5-6(N=26) and 6% at pH 4-5 (N=16). These drift responses are significantly less than those observed in

8

pH 4-5/[Al]>1 ppm and pH 5-6/[Al]>1 ppm, and are not different from cumulative drift in all other treatments and controls.

Mortality of Stenonema spp. did not differ significantly among the treatments (Fig. 1; G=7.69, df=5, p>0.10). However, mortality was highest when pH was <5 (18% and 10% for [Al]<1 ppm and [Al]>1 ppm respectively). Since drift was high in high acidity/high aluminum treatments, total population losses were greatest under these conditions. Again pH 5-6/[Al]>1 ppm and pH 4-5/[Al]>1 ppm treatments caused significantly greater population than all other treatments (G=53.82, df=5, p<<0.005).

Ephemerella subvaria (McDonnough)

As with Stenonema spp., cumulative drift of E. subvaria was significantly increased by acidity or aluminum additions. Cumulative drift at pH 5-6/[Al]>1 ppm and pH 6-7/[Al]>1 ppm (>25%) were significantly greater than cumulative drift in all other treatments and the control (<11%) (Fig. 2; G=25.42, df=5, p<0.005). These high drift rates were not due to high salinities, Cumulative drift in high $K_2(SO_4)$ at was 0% pH 6-7 (N=13) and 14% at pH5-6 (N=14). These drift rates are significantly less than cumulative drift at pH 6-7/[Al]>1 ppm and pH 5-6/[Al]>1 ppm, and do not differ from, or are less than, cumulative drift in the controls. Daily drift rates exceeded 10% only in high aluminum/low acidity treatments (Table 3). Thus, whereas Stenonema spp. drifted under conditions of high aluminum and moderate to high acidities, E. subvaria drifts under conditions of high aluminum and low to moderate acidities.

The highest mortality of E. subvaria was at pH 4-5/[Al]<1 ppm. Mortality at pH 4-5/[Al]<1 ppm was significantly greater than that in the control treatments (Fig. 2; G=10.98, compare to X^2=11.070, df=5, p=0.05).

Total population losses were higher at pH 5-6/[Al]>1 ppm than in controls (G=10.73, df=5, 0.10<p<0.05).

Ephemerella catawba (Traver)

Unlike the other two mayflies studied, cumulative drift of E. catawba was high in all treatments and the control and did not differ among the treatments (Fig. 3; G=8.71, df=5, p>0.10). Cumulative drift was highest under conditions of low pH and high aluminum (68% and 64% at pH 5-6/[Al]>1, and pH 4-5/[Al]>1 respectively). Population losses were especially high on the first day (Table 3). E. catawba is a very active mayfly with high drift rates in nature (Haney et al. 1983). High drift under control conditions obscured treatment effects.

Mortality of E. catawba differed significantly among the treatments (Fig. 3; G=13.40, df=4, p<0.01). The highest mortality (19%) at pH 4-5/[Al]>1 ppm. was significantly higher than all other treatments and the control, except treatments of pH 6-7/[Al]>1 ppm and pH 4-5/[Al]<1. The high mortalities in pH4-5/[Al]>1 ppm and pH6-7/[Al]>1 ppm were not due to the high salinity. There was no mortality in high $K_2(SO_4)$ treatments at both pH 4-5 (N=23) and pH6-7 (N=32). The total population losses at pH 4-5/[Al]>1 ppm were greater than total population losses in controls and treatments, except pH 5-6/[Al]>1 ppm or ph 4-5/[Al]<1 ppm (G=20.90, df=5, p<0.005).

Trichoptera

Hydropsyche betteni, H. ellisoma (Ross)

Hydropsyche spp. are unaffected by acid or aluminum alterations. Cumulative drift was less than 7% (Fig. 4), with daily drift rates less than 3% (Table 3). Differences could not be detected in cumulative drift rates

(G=5.04, df=5, p>0.10). Total population losses did not exceed 17% and were homogenous (G=7.66, df=5, p>0.10).

Macrostemum zebratum (Hagen)

Cumulative drift of M. zebratum differed significantly among treatments (Fig. 5; G=10.99, compare to X^2=11.070, df=5, p=0.05), but cumulative drift was highest at pH 4-5/[Al]<1 ppm (39%) and in controls (36%). Therefore increased acidity and aluminum did not increase drift responses. Drift losses were highest on the first day of the experiment (table 3), possibly inability to find a suitable substrate caused much of the drift.

Mortality of Macronema spp. increased at high aluminum concentrations, but was due to the high salinities. Mortality was significantly higher at pH 6-7/[Al]>1 ppm than in controls (Fig. 5; G=15.52, df=5, p<0.01). However, mortality was a result of the high salinities present in pH 6-7/[Al]>1 ppm treatments. Mortality in $K_2(SO_4)$ treatments at pH 6-7 (11%, N=26) did not differ significantly from mortality at pH 6-7/[Al]>1 ppm but was significantly greater than mortality in controls (p<0.05). While drift was highest in pH 4-5/[Al]<1 ppm and control treatments, mortality was lowest (3% and 2% respectively). Thus, total population losses did not differ significantly among the treatments (G=1.64, df=5, p>0.50).

Potamyia flava (Hagen)

This hydropsychid caddisfly was similar to Hydropsyche spp. in that drift was rare and cumulative drift did not differ among the treatments (Fig. 6; G=1.57, df=4, p>0.50). Like M. zebratum , P. flava daily drift rates were consistently higher on the first day of exposure (Table 3).

Mortality of P. flava was highest at pH 5-6/[Al]>1 ppm and pH 4-5/[Al]<1 ppm (16% and 21% respectively), differing significantly from controls and all

11

other treatments, except pH 6-7/[Al]>1 ppm (Fig. 6; G=21.90, df=5, p<0.005).

Mortality in the high aluminum treatments was caused by high salinities.

Mortality in treatments of high $K_2(SO_4)$ at pH 5-6 and pH 6-7 (15%, N=20; 17%,

N=23, respectively) were significantly greater than mortalities observed in

controls but did not differ from mortality at pH 6-7/[Al]>1 ppm and pH 5-

6/[Al]>1 ppm. Since drift was low in the two treatments with high mortality,

total population losses were not significantly higher in any of the

treatments. Mortality at pH 5-6/[Al]<1 ppm (3%) was significantly less than

all other treatments (G=26.22, df=5, p<0.005). The cause of this reduced

mortality is unknown. Chimarra obscurra (Walker)

Like P. flava, cumulative drift of the philopotamid C. obscurra was

unaffected by acidity or aluminum (Fig. 6; G=8.70, df=5, p>0.10). Daily drift

rates tended to be highest on the first day of the experiment (Table 3).

Mortality varied significantly among the treatments only because mortality at

pH 5-6/[Al]<1 ppm was very low (Fig. 6; G=17.84, df=5, p<0.005). No

treatments caused mortality that was significantly higher than that in

controls. The high and erratic mortality rates of C. obscurra are probably

the result of handling and disturbances in our system. This caddisfly is not

as robust as other insects tested and may have been damaged in capture or

transport. Usually all that remained of dead C. obscurra were head capsules,

suggesting that agonistic interactions with hydropsychids for net building

sites may have caused many C. obscurra deaths. Total population losses were

significantly lower at pH 5-6/[Al]<1 ppm (24%) than at all other

acidity/aluminum treatments except pH 6-7/[Al]>1 ppm and pH 5-6/[Al]>1 ppm

(G=16.85, df=5, p<0.005). Interestingly, trials at pH 5-6/[Al]<1 ppm also

resulted in the lowest total population losses of P. flava.

12

Psephenus herricki (DeKay)

The water penny beetle (P. herricki) showed a drift response similar to that observed in E. subvaria. Cumulative drift was significantly higher at pH 6-7/[Al]>1 ppm and pH 5-6/[Al]>1 ppm (39% and 34% respectively) than in controls and pH 4-5/[Al]<1 ppm (G=17.60, df=5, p<0.001). However, unlike E. subvaria, responses were due to high salinities in low acidity/high aluminum treatments. Cumulative drift in high $K_2(SO_4)$ at pH 5-6 (30%, N=18) and pH 6-7 (45%, N=20) was not significantly different from cumulative drift at pH 5-6/[Al]>1 ppm and pH 6-7/[Al]>1 ppm, but was significantly greater than cumulative drift in controls. Daily drift rates exceeded 10% only in the three treatments with aluminum concentration greater than 1 ppm (Table 3), indeed, daily drift rates greater than 20% are observed under conditions of pH 6-7/[Al]>1 ppm and pH 5-6/[Al]>1 ppm.

Mortality under conditions of pH 5-6/[Al]<1 ppm (1%) was significantly less than mortalities observed in all other treatments, but did not differ from mortality observed in controls (4%) (Fig. 7; G=17.02, df=5, p<0.001). Increased mortality did not result from increased acidity or aluminum concentrations.

Total population losses in treatments with aluminum concentrations of >1 ppm were significantly higher than those with aluminum concentrations of <1 ppm (Fig. 7; G=28.51, df=5, p<0.001). However, as already stated, the response of P. herricki was due to the salinities present in high aluminum treatments.

Amphinemura nigritta (Provancher)

Drift of A. nigritta was rare and cumulative drift did not differ significantly among the treatments (Fig. 8; G=3.02, df=4, p>0.50). Daily

13

drift rates (Table 3) further show that drift rates of A. nigritta were very low, generally 5% or less.

Treatments affected mortality more than drift, but significant differences are still lacking (Fig. 8; G=7.17, df=4, p>0.10). Maximum mortality occured at at pH 6-7/[Al]>1 ppm and pH 4-5/[Al]<1 ppm (20% and 17% respectively), the same conditions that produced the highest cumulative drift (10% and 11% respectively).

Because drift and mortality were highest under the same conditions, total population losses were significantly higher at pH 6-7/[Al]>1 ppm (28%) and pH 4-5/[Al]<1 ppm (28%) than in controls (Fig. 8; G=17.36, df=5, p<0.01), and was not due to high salinity. Total population losses in high $K_2(SO_4)$ at pH 6-7 (6%) did not differ significantly from total population loss in controls, but did differ from total population loss at pH 6-7/[Al]>1 ppm.

Molt Success

Increased acidity reduced molt success, molting success was significantly higher under control conditions (95%) than at pH 5-6/[Al]<1 ppm (60%) and pH 4-5/[Al]<1 ppm (64%) (Fig. 9; G=18.94, df=5, p<0.005). Unsuccessful molts resulted from the inability of the molting insect to shed the cuticle. Only 4% (N=26) of molting failures observed in treatments were due to mortality prior to repigmentation following ecdysis. Apparently high acidity reduces the ability of the insects to shed the cuticle, rather than increasing their mortality following ecdysis. It is unclear why increased aluminum concentrations mediates this effect.

DISCUSSION

The objective of this experiment was to discover if short term acid precipitation events, such as spring snow melt, acutely affect stream

14

insects. Insects not found to be acutely affected by increased acidity or aluminum should not necessarily be considered to be unaffected by altered chemical conditions. Direct effects of pH on the physiology of insects are well documented. Hydrogen ions cause ionic imbalances by disrupting sodium/chloride regulation in aquatic insects (Stobart, 1967; 1971; Wright, 1975; van Genechten and van Puymbroek, 1980). Aquatic crustacea also show ionic imbalances when exposed to high acidities (Potts and Fryer, 1979; Malley, 1980; Morgan and McMahon, 1982; Havas and Hutchinson, 1983). It is possible that in this study, neither the 72 hour exposure period was not long enough, nor the number of individuals tested large enough, to detect hydrogen ion effects in many insects tested.

Longer exposure times might also increase drifting behavior of insects that showed no acute drifting response. Psephenus herricki was chosen to test the long term effects of increased acidity upon drift because of its low mortality under conditions of pH 5-6/[Al]<1. As in the short term exposure treatments, cumulative drift following three days exposure did not differ between controls and treatments of pH 5-6 with no aluminum additions (aluminum was not added to these long term treatments to prevent any salinity effects upon drifting behavior)(Fig 10; G=3.17, df=1, p>0.05). Mortality following ten days exposure was low and did not differ significantly between controls (8%) and treatments (4%)(G=0.69, df=1, p>0.10), but cumulative drift was significantly higher than in controls (Fig. 10; G=5.78, df=1, p<0.05). Thus, long term pH depression may cause increased drifting behavior of stream insects, even if mortality of the insects is not affected.

Longer exposure periods may show more effects of acidity and aluminum upon stream insects, but, artifacts may be introduced by long term exposure under laboratory conditions. A three day exposure period reduced the amount

15

of mortality due to bacterial or fungal infections. In our study, many of the insects killed by the treatment were colonized by fungi after only three days exposure. A three day exposure period also avoided complications due to reduced food availability to the insects. While algae was present on rocks and trough sides, and organic matter was present in the water, food levels were not as high as those in the field and long term exposure would reduce food levels resulting in stressed test insects. Thus, while effects present only when exposure time is long were missed, observed effects could be ascribed to the altered chemical conditions.

Effects of Aluminum

Aluminum leaching into aquatic systems is a major cause of fish mortality (Driscoll et al. 1980, Muniz and Leivestad 1980). The mortality is due to the formation of a yellow mucus on the gills which interferes with respiration and ionoregulation and causes physiological stress. We examined the gills of insects subjected to high aluminum concentrations, but a mucus coating could not be detected. In only one of the insects tested (Ephemerella catawba) did aluminum contribute to mortality. In other species mortality was due to increased salinities in high aluminum treatments (M. zebratum spp. and Potamyia flava), or was high, but not significant in high aluminum treatments (Amphinemoura nigritta). The cause of insect mortality in high aluminum conditions does not appear to be due to the formation of a mucus on the gills, as is the case with fish mortality under high aluminum conditions.

In this study, the major effect of aluminum upon stream insects was the increased drifting behavior of Ephemeroptera. Both Ephemerella subvaria and Stenonema modestum increased their drift rates with increased aluminum concentrations. These responses were not caused by salinities in these

16

treatments. <u>Stenonema</u> <u>modestum</u> drifted in response to high aluminum concentrations at high to moderate acidities (pH 4-6), whereas <u>E.</u> <u>subvaria</u> drifted in response to high aluminum concentrations at low to moderate acidities (pH 5-7). We can infer that these two species were affected by different forms of aluminum since pH regulates aluminum speciation (Hem and Roberson 1967, Roberson and Hem 1969). Aluminum forms octahedral complexes in aqueous solutions involving water, hydroxide, fluoride of sulfate ions. At neutral pH, hydroxide complexes dominate; hydroxides of aluminum would thus be affecting <u>E.</u> <u>subvaria</u> in treatments of pH6-7. Under acidic conditions (pH<5) hydroxide complexes are reduced, and since fluoride is present at low concentrations in Oyster River water, organic and/or sulphate complexes should be the form of aluminum affecting <u>Stenonema</u> spp. in low pH treatments.

The high organic content of the Oyster River water and the presence of algae on the rocks and trough sides may also have influenced aluminum speciation. Organic molecules form strong complexes with aluminum (Lind and Hem 1975). Fish mortality due to high aluminum levels is reduced if citrate is added (Driscoll et al. 1980). The high organic content of the Oyster River water may have reduced mortalities in high aluminum treatments.

Since aluminum was not found to cause significantly higher mortality or drift response of Trichoptera and <u>Psephenus</u> <u>herricki</u> aluminum leaching into watersheds due to acid precipitation should not cause large population losses of these insects. In New Hampshire, aluminum concentrations of 0.6 ppm have been measured (Johnson et al. 1981), but are high. In this experiment, high aluminum concentrations were 2-3 times greater than this value. Aluminum concentrations higher than those tested in this experiment have been observed in acid mine drainage (Peckarsky and Cook 1981), but it is unlikely that

17

leaching due to acid precipitation could cause aluminum concentrations to reach levels capable of affecting P. herricki or the trichopterans tested.

Effects of Acidity

Ephemeroptera were more affected by reduced pH than are the other insects tested. Acidity increased mortality, but did not greatly increase drift by mayflies. Although mortality was not significantly higher in Stenonema modestum and the stonefly, Amphinemoura nigritta, all mayflies and the stonefly had highest mortality at pH<5. Of the trichopterans, only P. flava had increased mortality when exposed to high acidities. Other trichopterans had higher mortality due to increased salinity in the test water (Macronema spp.) and perhaps handling or competition (C. obscurra), or showed very little mortality (Hydropsyche spp.).

Ionic imbalances are found in insects exposed to high acidities (Stobbart, 1967; 1971; Wright, 1975; van Genechten and van Puymbroek, 1980), thus suggesting an explanation for the reduction in mortality when $AlK(SO_4)$ was added to high acidity treatments. Except for E. catawba, all insects showing high mortalities under acidic conditions had lower mortality when aluminum concentrations were raised above 1 ppm. The increased ionic concentration in high aluminum treatments may have facilitated ionic regulation under acidic conditions. Consistent with this idea, McDonald et al. (1980) showed that additions of calcium ions to low pH water reduced mortality in rainbow trout.

The observed increased molt failure with increased acidities is consistent with the laboratory study of Bell (1971), who found that molt failure was on important component of insect mortality in high acidity conditions. Also, Hall et al. (1980) found that insect emergence was reduced 37% following the acidification of a New Hampshire stream. Almost certainly,

18

longer exposure to low pH should increase mortality due to molt failure as more insects molt. Not all insects tested attempted to molt during the three day period (e. g. no trichopterans were observed to molt). In order to test the molting failure of these insects it would be neccessary to lengthen exposure period or collect insects that are preparing to molt.

Sanderson et al. (1981) found that in vitro additions of aluminum inhibited chromosomal puffing (associated with RNA synthesis) at ecdysterone activated sites on the polytene chromosome of _Simulium vittatum_. Since ecdysterone is an important molting hormone in insects, one might postulate that aluminum would adversely influence molting ability. Rather than increasing molt failure, we found that increased aluminum concentrations consistently increased molting success at high acidities. Thus, it appears either the aluminum effects on _Simulium_ do not apply to insects used in this study, or such effects require longer exposure to be seen in the molting response.

DIRECT OBSERVATIONS OF INSECT ACTIVITY

Our previous experiments clearly demonstrated the importance of drift as a measure of sublethal effects of lowered pH and elevated aluminum concentration. For certain insects such as the mayflies, significantly higher drift rates occurred with alterations in water chemistry that could not be detected in the short-term mortality. The drift behavior of mayflies and their related responses to changes in light intensity follow a well-defined pattern. Thus, we set out to develop a system to use direct measures of the activity of mayflies as a means of detecting changes in drift-related behavior. In this section we describe the experimental design

and testing of a time-lapse, infrared video system used to observe directly drift activities in the model streams.

EXPERIMENTAL SYSTEM

Video taping was conducted underneath the model streams by focusing the video camera on the underside of selected rocks (Fig. 11). Viewing ports were positioned under each rock to be observed. The video camera (Daage model MTI) was equipped with an infrared sensitive photocell so that recordings could be made during the night with illumination from a 15 watt lamp and color filter that cutoff light wavelengths below 750 nm. Since insects have no pigments active at or above 750 nm, observations were made with no visual disturbance to the mayflies. Video recordings were made with a Gyyr Time Lapse Recorder, using 0.5 inch VHS video tapes. Recordings were made at speeds that compressed real time either 24 or 48 times, i.e. 24 hours were reduced to either 1 or 0.5 hours. Activities were recorded continuously for three to five day experiments. Light intensity was measured throughout each experiment using an International Light Research Radiometer Model IL-700 with a SEA-400 photocell. Light measurements were recorded automatically with a Li-Cor Printing Integrator at 10 minute intervals.

Experiments were conducted in the AFAIR laboratory. Large south-facing windows provide direct sunlight to portions of the model stream at midday and a natural photoperiod. Temperature was controlled with an immersion cooler placed in the reservoir of the stream (Fig. 12).

Animals were collected from either the Oyster River or Bellamy River and placed in the model stream one day before the start of the experiment. Approximately 10-15 heptageniid mayflies were placed on each observation

20

rock. Model stream temperature was adjusted to the temperature of natural stream at midday.

ANALYSIS OF THE VIDEO TAPES

Video tapes were placed back at 24-48 times real time for analysis. Images were displayed on a 21 or 25 inch monitor. Tracings were then made of the movements of each insect for 10 minute periods, using transparent plastic overlays. From cartometer measurements of these tracings, distances travelled and locomotor activity rates (distance travelled per unit time) were calculated at various times over the diel cycle. Counts were also made of the number of insects on the underside of the rock, since drift behavior is generally preceded by a movement of animals to the upperside of the substrate.

RESULTS

Experiments were conducted to examine the light control of mayfly locomotor activity and the movement of animals from underneath the rocks, using the baetid mayfly, *Stenonema* *modestum*. Variations in weather conditions provided a range of about four orders in magnitude of absolute light intensity at the time of onset of evening activity.

Some representative patterns of evening activity and movement of *Stenonema* are shown in Figs. 13-16. Locomotor activity was consistently low and numbers of animals under the rocks consistently high throughout the day leading up to the period depicted in the Figures. Although there was usually a decrease in abundance of animals under the rocks at dusk, activity rates were a much more reliable and sensitive indicator of the shift to the nocturnal mode. To test the light regulation model of Haney et al. (1983), estimates were made of the time of initiation of evening activity (IET-t) as

21

well as the time at which the light stimulus threshold proposed by Ringelberg (1964) (RS-t). The light regulation model proposes that the relative light change acts as a stimulus or zeitgeber at the time it reaches a critical threshold value (RS=0.0017/sec). The drift response occurs after some delay in time which is determined by the light intensity at the time of activitation by the light stimulus (RS-t). The same general pattern of light control appears to apply to _Stenonema_ _modestum_, although the response parameters measured in this study are not the same as in Haney et al. (1983), where drift rates were measured. Using locomotor activity rates as an analog of drift in the model, a striking similarity is seen in the final regression model (Fig. 16). The initiation of evening locomotor activity is delayed up to about 30 min when the light intensity at dusk is high, whereas the nocturnal activity begins much earlier when evening light intensities are lower. Since at very low light _Stenonema_ activity began before the Ringelberg stimulus was achieved, the threshold stimulus value for _Stenonema_ activity must be considerably lower than the value of Ringelberg that we arbitrarily applied here simply as a reference point in time.

These preliminary results that direct video observation of insect activity can be used to examine drift related behavior. Observations can be quantified to permit an analysis of factors regulating drift such as the light regulation demonstrated here. Further experiments are needed to adjust the light model to the activities measured with this system, such as definition of the light stimulus threshold value for the study organism and the effect of absolute light intensity on this value. Test species would then be subjected to changes in water chemistry. The effect of these perturbations on the light control model can then be evaluated.

SUMMARY AND CONCLUSIONS

Ephemeropterans were strongly affected by increased acidity and aluminum concentrations. Cumulative drift of Stenonema spp. and Ephemerella subvaria were increased by aluminum, but drift of the two species were in response to aluminum at different acidities. Ephemerella subvaria was affected by high aluminum concentrations at low-moderate acidities when hydroxides are the dominant form of aluminum. Stenonema spp. was affected by high aluminum concentrations at moderate-high acidities when aluminum is complexed with ions other than hydroxide. A third mayfly, E. catawba, had high drift rates in all treatments. All mayflies had consistently higher mortality at pH<5.

The stonefly, Amphinemoura nigritta, also had high mortality at pH<5, but significantly higher responses were found only in total population loss rates at pH 6-7/[Al]>1 ppm and pH 4-5/[Al]<1 ppm. Effects of aluminum upon drift rates in mayflies and upon total population losses in A. nigritta were not due to the high salinities in aluminum treatments, however, increased salinity of high aluminum treatments reduced mortality in high acidity treatments.

The caddisflies tested showed no significant responses to increased aluminum concentrations but did respond to the high salinities in these treatments. Hydropsyche spp. responses were consistently low and and not significant. Macronema spp. mortality was increased by high salinities in low acidity-high aluminum treatments. Potamyia flava had low drift rates, but had high mortality at pH 4-5/[Al]<1 ppm and in high salinity treatments at pH 5-6. Chimarra obscurra suffered high mortality under all treatment conditions except pH 5-6/[Al]<1 ppm suggesting that mortality was due to agonistic interactions or improper handling. High drift rates of

23

caddisflies on the first day of the experiment suggested that many individuals drifted because they could not find an appropriate substrate.

Drift of the water penny beetle, _Psephenus herricki_, was increased by high salinities but mortality was unaffected. Long term exposure of _P. herricki_ to high acidities increased drift but not mortality. Molt failure was increased by high acidities.

ACKNOWLEDGEMENTS

CITATIONS

Bell, H. L. and A. V. Nebeker. 1969. Preliminary studies on the tolerance of aquatic insects to low pH. J. Kansas Ent. Soc. 42: 230-236.

----------. 1971. The effects of low pH on the survival and emergence of aquatic insects. Water Res. 5: 313-319.

Brigham, A. R., W. U. Brigham and A. Gnilka (eds.). 1982. Aquatic insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterp. Mahomet Ill.

Cronan, C. S. and C. L. Schofield. 1979. Aluminum leaching response to acid precipitation: effects on high-elevation watersheds in the Northeast. Science. 204: 304-306.

Driscoll, C. T., J. P. Baker, J. J. Bisogni Jr. and C. L. Schofield. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature. 284: 161-164.

Hall, R. J., G. E. Likens, S. B. Fiance and G. R. Hendry. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology. 61: 976-989.

Haney, J. F., T. R. Beaulieu, R. P. Berry, D. P. Mason, C. R. Miner, E. S. McLean, K. L. Price, M. A. Trout, R. A. Vinton and S. J. Weiss. 1983. Light intensity and relative light changes as factors regulating stream drift. Arch. Hydrobiol. 97: 73-88.

Harriman, R. and B. R. S. Morrison. 1982. Ecology of streams draining forested and non forested catchements in an area of central Scotland, UK, subject to acid precipitation. Hydrobiol. 88: 251-264.

Havas, M. and T. C. Hutchinson. 1982. Aquatic invertebrates from the Smoking Hills, Northwest Territories: effect of pH and metals on mortality. Can. J. Fish. Aquatic Sci. 39: 890-903.

-------------------------------------. 1983. Effects of low pH on the chemical composition of aquatic invertebrates from tundra ponds at the Smoking Hills N.W.T. Canada. Can. J. Zool. 61: 241-249.

Hem, J. D. and C. E Roberson. 1967. Form and stability of aluminum hydroxide complexes in dilute solution. U. S. G. S. Water Supply Paper #1827-a. U. S. Gov't Printing Off. Washington DC.

Hitchcock, S. W. 1974. The Plecoptera or Stoneflies of Connecticut. State Geol. Nat. Hist. Survey of Conn. Bull. 107 262 pgs.

Johnson, N. M., C. T. Driscoll, J. S. Eaton, G. E. Likens and W. H. McDowell. 1981. Acid rain, dissolved aluminum and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire, USA. Geochim. Cosmochim. Acta. 45: 1421-1438.

Koryak, M., M. A. Shapiro and J. L. Sykora. 1972. Riffle zoobenthos in streams recieving acid mine drainage. Water Res. 6: 1239-1247.

Likens, G. E. and F. H. Bormann. 1974. Acid rain: a serious environmental problem. Science. 184: 1176-1179.

Lind, C. J. and J. D. Hem. 1975. Effects of organic solutes on chemical reactions of aluminum. U. S. G. S. Water Supply Paper #1827-g. U. S. Gov't Printing Off. Washington DC.

Malley, D. F.. 1980. Decreased survival and calcium uptake by the crayfish, Procambarus virilis in low pH. Can. J. Fish. Aquatic Sci.. 37: 364-372.

McDonald, D., H. Hobe and C. Wood. 1980. The influence of calcium on the physiological responses of the rainbow trout, Salmo gardneiri, to low environmental pH. J. Exp. Biol. 88: 109-131.

Morgan, D. O. and B. R. McMahon. 1982. Acid tolerance and effects of sublethal acid exposure of iono-regulation and acid-base status in 2 crayfish, Procambarus clarki and Orconectes rusticus. J. Exp. Biol. 97: 241-252.

Muniz, I. P. and M. Leivestad. 1980. Toxic effects of aluminum on the brown trout, Salmo trutta. In: Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. SNSF Project, Norway. Editors: D. Drablos and A. Tollan. pgs. 320-321.

Peckarsky, B. L. and K. Z. Cook. 1981. Effects of Keystone Mine effluent on colonization of stream benthos. Environ. Entomol. 10: 864-871.

Potts, W. T. W. and G. Fryer. 1979. The effects of pH and salt concentration on sodium balance in Daphnia magna and Acantholeberis curvirostris. J. Comp. Physiol. 129: 289-294.

Rainwater, F. H. and L. L. Thatcher. 1960. Methods of Collection and Analysis of Water Samples. U. S. Geol. Surv. Water Supply Paper 1454. U. S. Govt. Printing Off. Washington DC.

Roberson, C. E. and J. D. Hem. 1969. Solubility of aluminum in the presence of hydroxide, fluoride and sulfate. U. S. G. S. Water Supply Paper #1827-c. U. S. Gov't Printing Off. Washington DC.

Ross, H. H. 1944. The Caddisflies or Trichoptera of Illinois. Ill. Nat. Hist. Survey Bull. 23: 1-326.

Sanderson, C. L., D. R. McLachlan and U. deBoni. 1982. Altered steroid induced puffing by chromatin bound aluminum in a polytene chromosome of the blackfly Simulium vittatum. Can. J. Gen. Cyt. 24: 27-36.

Stobbart, R. H. 1967. The effect of some anions and cations upon the fluxes and net uptake of chlorides in the larva of Aedes aegypti, and the nature of the uptake mechanisms for sodium and chloride. J. Exp. Biol. 47: 35-57.

------------------. 1971. Evidence for Na^+/H^+ and Cl^-/HCO_3^- exchanges during independent sodium and chloride uptake by the larva of the mosquito _Aedes aegypti_. J. Exp. Biol. 54: 19-27.

Webb, A. H. 1982. Weak acid concentrations and river chemistry in the Tovdal River, southern Norway. Water Res. 16: 641-648.

Winner, R. W., M. W. Boesel and M. P. Farrell. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can. J. Fish. Aquatic Sci. 37: 647-655.

Wright, D. A. 1975. The effects of external sodium concentration upon sodium fluxes in _Chironomus dorsalis_ and _Camptochironomus tentans_ and the effect of other ions on sodium influx in _C. tentans_. J. Exp. Biol. 62: 141-155.

vanGenechten, J. H. D., S. vanPuymbroek and O. L. J. vanDerborght. 1980. Effect of pH on the chloride uptake and efflux in 2 waterbugs from acid freshwaters. Comp. Biochem. Physiol. A Comp. Physiol. 67: 85-90.

TABLE 1: Descriptive statistics of sites providing insects tested.

river	site	river size (M) widthXdepth	test insect found at site	density of test insect at site (#/M^2) aver.	N	max.	date
Oyster	1	4 X 0.25	S. modestum	86	3	118	4/4/85
"	2	7 X 0.50	E. subvaria	84	3	106	3/25/85
"	3	8 X 0.40	Hydropsyche	360	3	478	8/18/83
			M. zebratum	240	3	341	"
			other Hydropsychidae*	366	3	493	"
			C. obscurra	58	3	88	"
North	1	8 X 0.40	P. herricki	136	3	161	4/4/85
"	2	7 X 0.30	E. catawba	276	3	344	"
			A. nigratta	93	3	108	"

*includes P. flava, Cheumatopsyche and Parapsyche

Table 2: Descriptive statistics on insect densities (#/M^2) in artificial streams.

test insect	average	maximum	minimum
S. modestum	39.7	86.1	2.7
E. subvaria	29.7	77.7	2.7
E. catawba	42.7	88.9	11.1
Hydropsyche spp.	106.6	172.2	66.7
M. zebratum	31.9	61.1	16.7
P. flava	41.2	97.2	13.9
C. obscurra	38.9	66.7	16.7
A. nigritta	32.3	72.6	2.7
P. herricki	32.4	78.0	8.3

TABLE 3: average rates of daily population change due to drift out of troughs (%/day).

	CONTROL	pH 6-7 [Al]>1	pH 5-6 [Al]<1	pH 5-6 [Al]>1	pH 4-5 [Al]<1	pH 4-5 [Al]>1
			S. modestum			
DAY 1	4.3	1.0	5.9	8.8	2.6	12.3
DAY 2	0.8	0.0	0.0	9.6	5.4	11.2
DAY 3	0.0	2.0	3.1	12.1	0.0	7.9
average	1.7	1.0	2.9	10.2	2.7	10.5
			Ephemerella subvaria			
DAY 1	4.9	10.7	0.0	2.7	0.0	6.4
DAY 2	0.0	0.0	8.5	22.2	0.0	3.9
DAY 3	1.3	16.0	2.3	17.9	3.3	1.0
average	2.1	8.9	3.6	14.3	1.1	3.8

Ephemerella catawba

DAY 1	32.5	28.9	42.1	51.0	37.5	48.4
DAY 2	11.5	20.3	9.1	12.0	20.0	21.9
DAY 3	13.0	10.6	20.0	27.3	12.5	12.0
average	19.0	19.9	23.7	30.1	23.3	27.4

Hydropsyche spp.

DAY 1	1.0	2.6	1.6	1.0	1.8	0.7
DAY 2	3.0	2.1	0.0	1.6	0.0	0.9
DAY 3	1.0	1.6	1.2	2.7	0.9	2.1
average	1.7	2.1	0.9	1.8	0.9	1.2

M. zebratum

DAY 1	26.9	17.4	15.5	17.7	27.3	18.3
DAY 2	6.6	2.6	0.0	0.0	4.2	2.1
DAY 3	5.6	2.7	5.6	3.9	13.0	3.3
average	13.0	6.8	7.0	7.2	14.8	7.9

Potamyia flava

DAY 1	5.6	7.3	0.0	6.0	4.3	3.9
DAY 2	1.5	2.0	0.0	0.0	0.0	0.6
DAY 3	1.5	0.0	0.0	0.0	0.0	1.8
average	2.9	3.1	0.0	2.0	1.4	2.1

Chimarra obscurra

DAY 1	16.1	10.2	13.2	3.0	11.6	6.9
DAY 2	3.0	1.9	0.0	3.1	2.6	6.0
DAY 3	3.1	1.9	2.0	0.0	2.7	1.4
average	7.4	4.7	5.1	2.0	5.6	4.8

Psephenus herricki

DAY 1	7.5	27.8	8.5	6.3	7.7	15.7
DAY 2	5.4	7.7	6.8	23.3	0.0	9.3
DAY 3	2.9	8.3	2.8	8.7	0.0	7.7
average	5.3	14.6	6.0	12.8	2.6	10.9

Amphinemoura nigratta

DAY 1	2.6	2.8	14.3	0.0	0.0	1.9
DAY 2	0.0	1.4	0.0	0.0	5.5	3.8
DAY 3	0.0	4.4	0.0	0.0	5.9	0.0
average	0.9	2.9	4.8	0.0	3.8	1.9

Figure 1. Responses of <u>Stenonema</u> <u>modestum</u> to treatments. Drift following

first day represented by rightward slanting lines; drift from second day

represented by leftward slanting lines; drift from third day represented by

open rectangle; mortality is represented by filled rectangle. Letter beside

bar indicates homogenous sets, responses that form homogenous sets at $p<0.05$

are assigned the same letter; lower case letters represent associations among

cumulative drift, capital letters represent mortality associations. Asterix

above bar indicates total population losses that significantly differ from

controls.

Figure 2. Responses of <u>Ephemerella</u> <u>subvaria</u> to treatments. Symbols used are

the same those as in figure 1.

Figure 3. Responses of <u>Ephemerella</u> <u>catawba</u> to treatments. Symbols used are

the same as those in figure 1.

Figure 4. Responses of <u>Hydropsyche</u> spp. to treatments. Symbols used are the

same as those in figure 1.

Figure 5. Responses of <u>Macrostemum</u> <u>zebratum</u> to treatments. Symbols used are

the same as those in figure 1.

Figure 6. Responses of <u>Potamyia</u> <u>flava</u> to treatments. Symbols used are the

same as those in figure 1.

Figure 7. Responses of <u>Chimarra</u> <u>obscurra</u> to treatments. Symbols used are

the same as those in figure 1.

Figure 8. Responses of <u>Psephenus</u> <u>herricki</u> to treatments. Symbols used are

the same as those in figure 1.

Figure 9. Responses of <u>Amphinemoura</u> <u>nigritta</u> to treatments. Symbols used

are the same as those in figure 1.

Figure 10. Long-term drift responses of <u>Psephenus</u> <u>herricki</u> to lowered pH.

Figure 11. Placement of the infra-red lamp and video camera under the experimental stream for viewing the activity of insects on the underside of the rocks.

Figure 12. Arrangement of experimental stream and recording laboratory in the Anadromous Fish and Aquatic Invertebrate Research facility.

Figure 13. Patterns of locomotor activity (●) and number of Stenonema modestum (✗) on the underside of the rocks.

Figure 14. As in Figure 13.

Figure 15. As in Figure 13.

Figure 16. Relationship between the time delay for initiation of increased evening locomotor activity ($IEA_t - RS_t$) and the light intensity at the time of the threshold light stimulus (IRS_t). See text for explanation of terms.
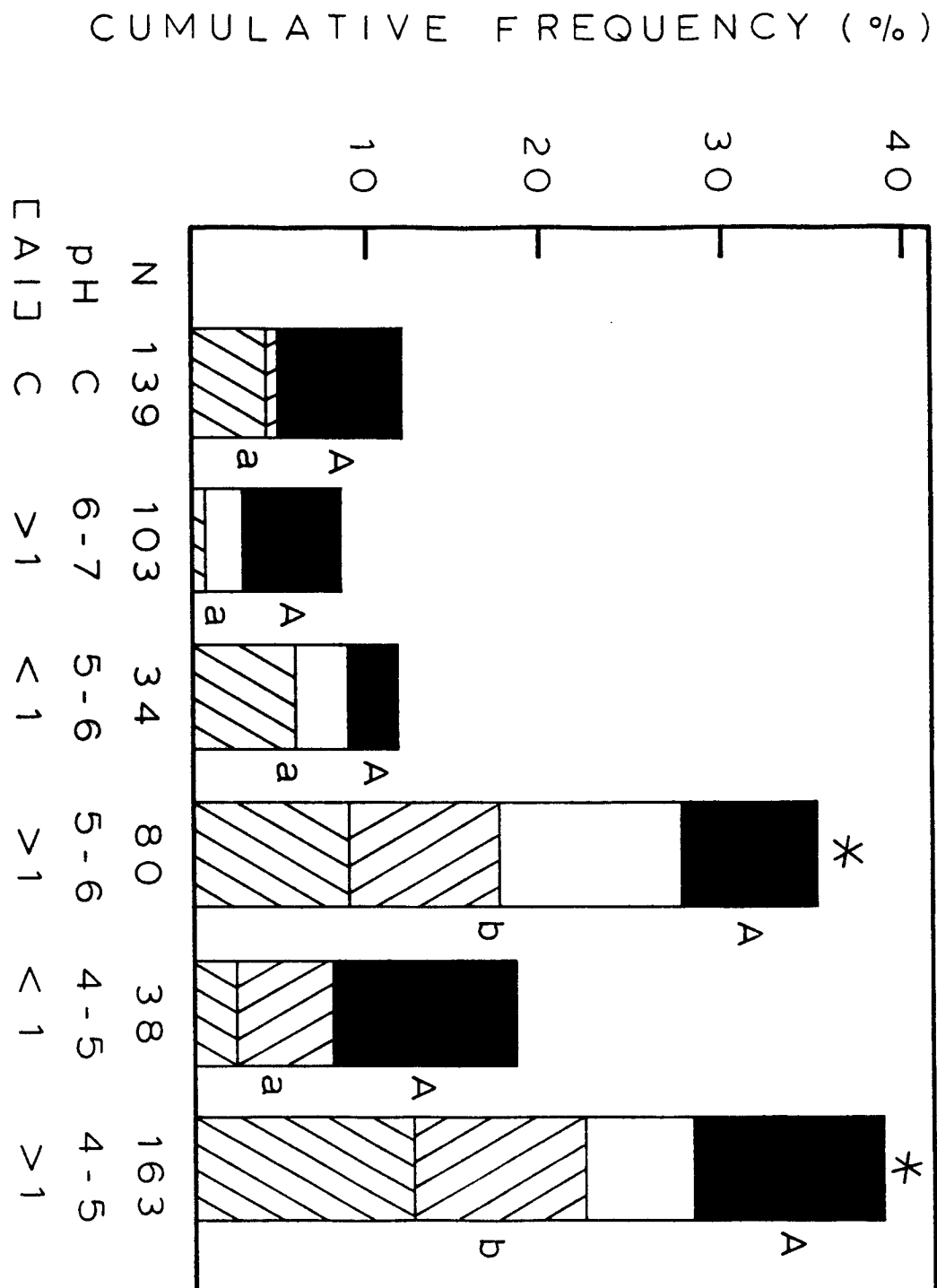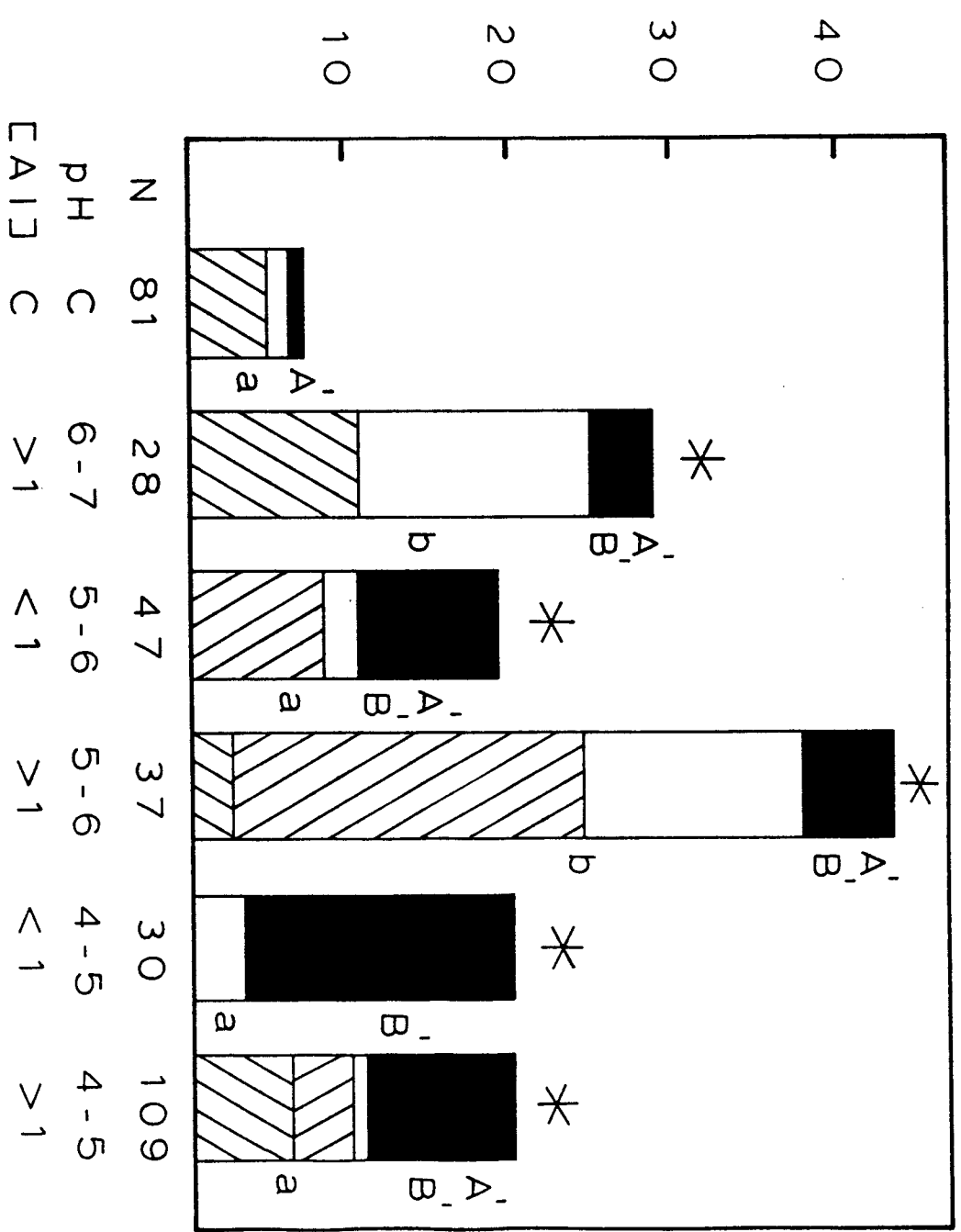
CUMULATIVE FREQUENCY (%)

FIGURE 1

FIGURE 2

FIGURE 3

CUMULATIVE FREQUENCY (%)

FIGURE 4

N	311	193	251	189	113	439
pH	C	6-7	5-6	5-6	4-5	4-5
[Al]	C	>1	<1	>1	<1	>1

FIGURE 5

CUMULATIVE FREQUENCY (%)

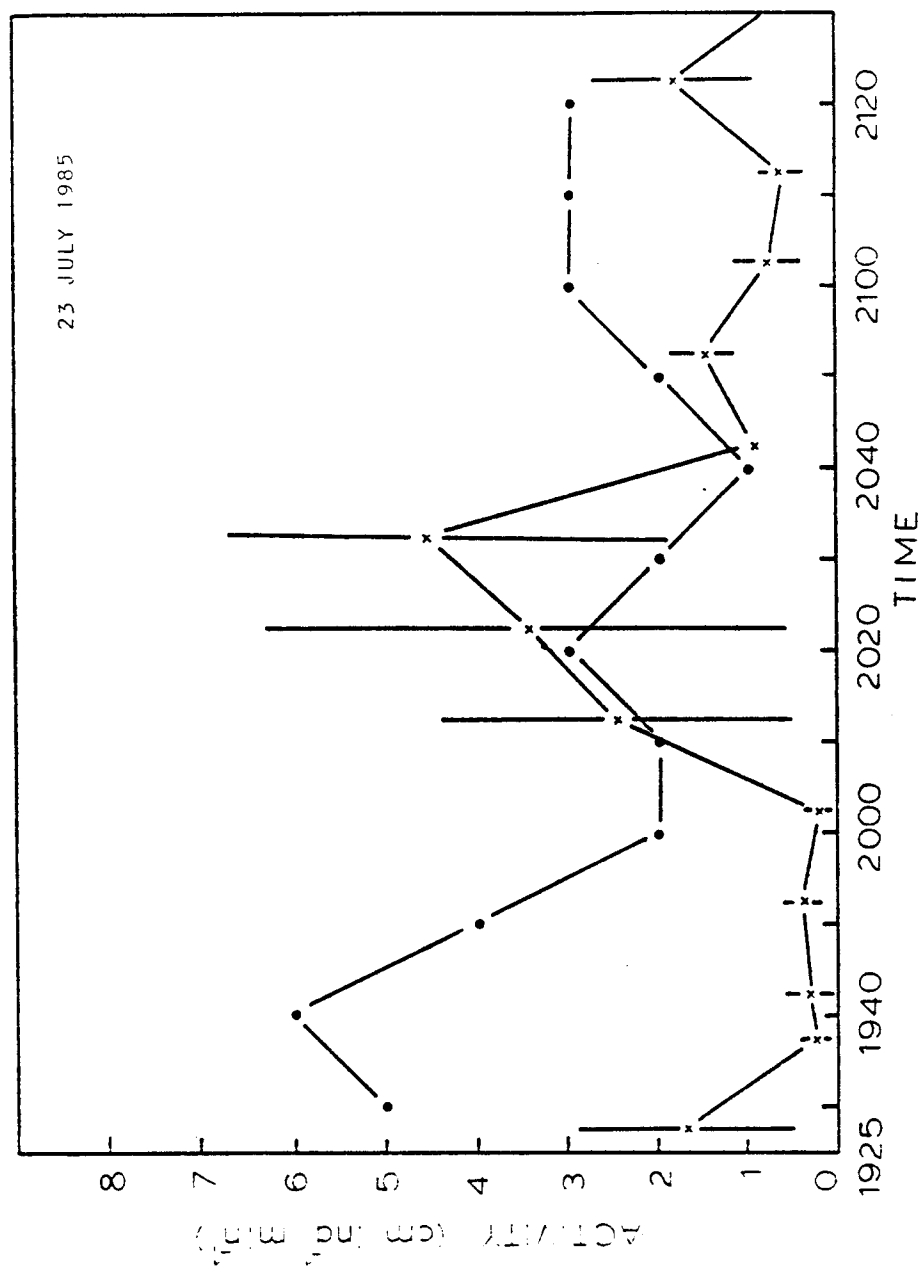| N | 144 | 55 | 105 | 50 | 46 | 179 |
| pH | C | 6-7 | 5-6 | 5-6 | 4-5 | 4-5 |
| [Al] C | C | >1 | <1 | >1 | <1 | >1 |
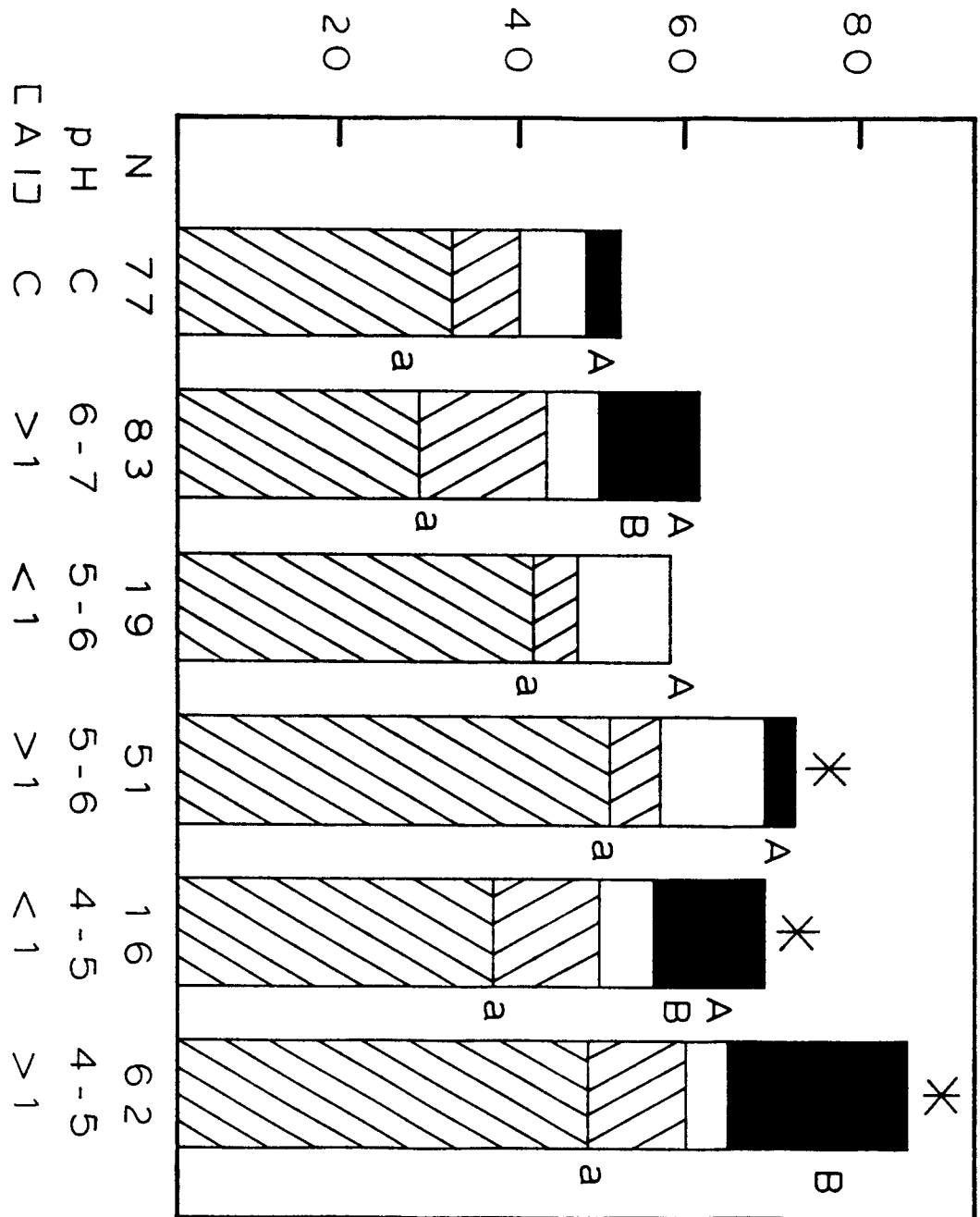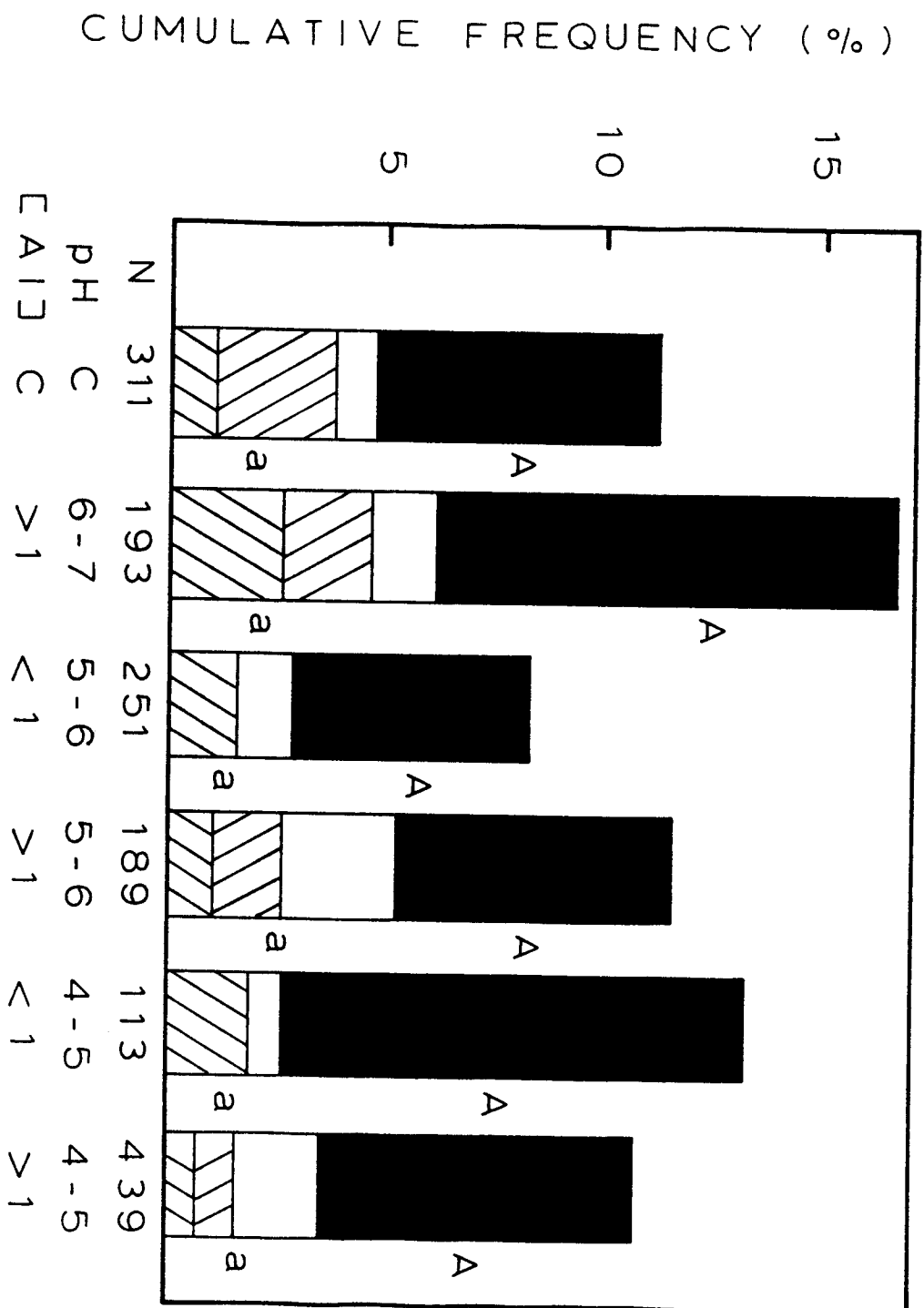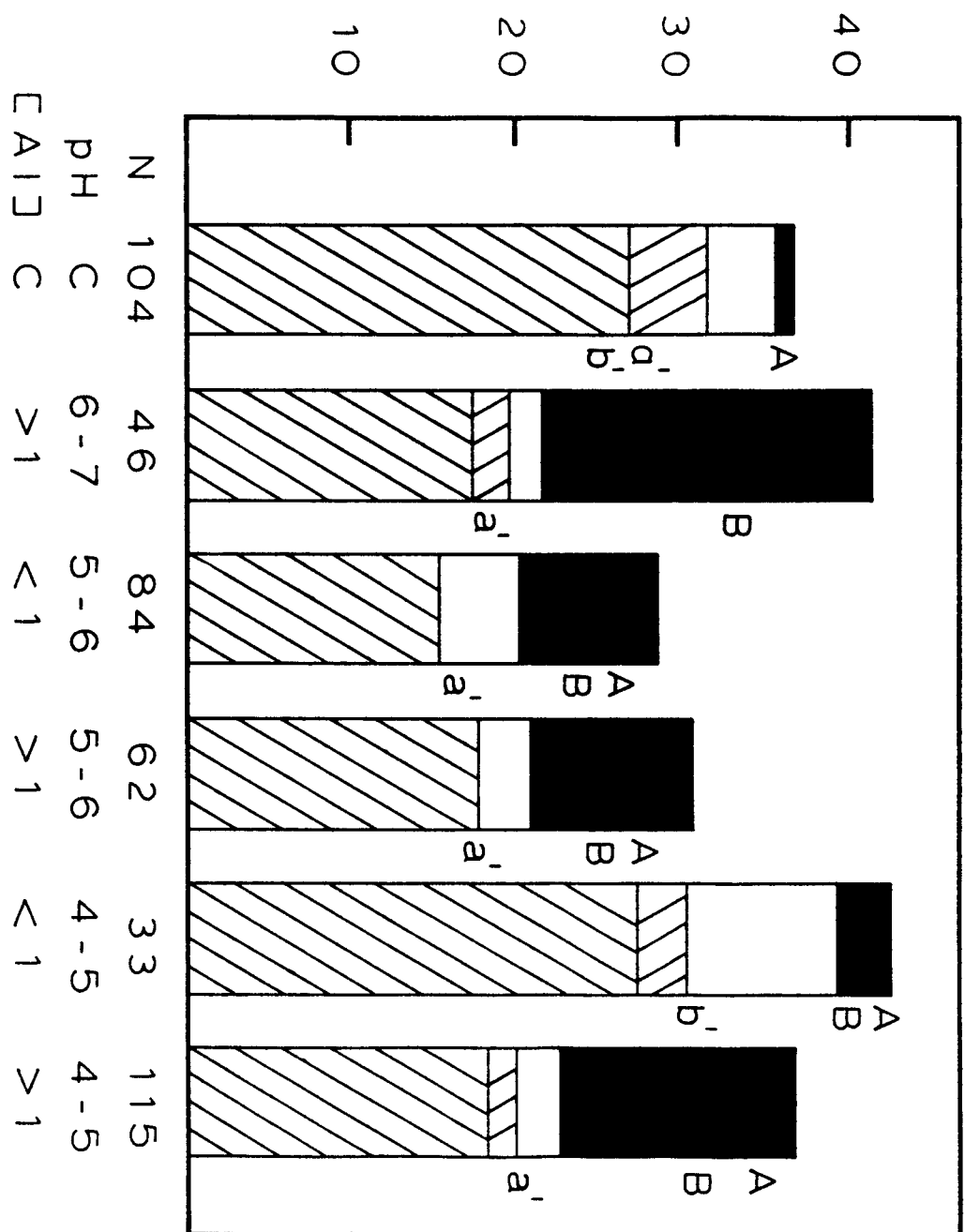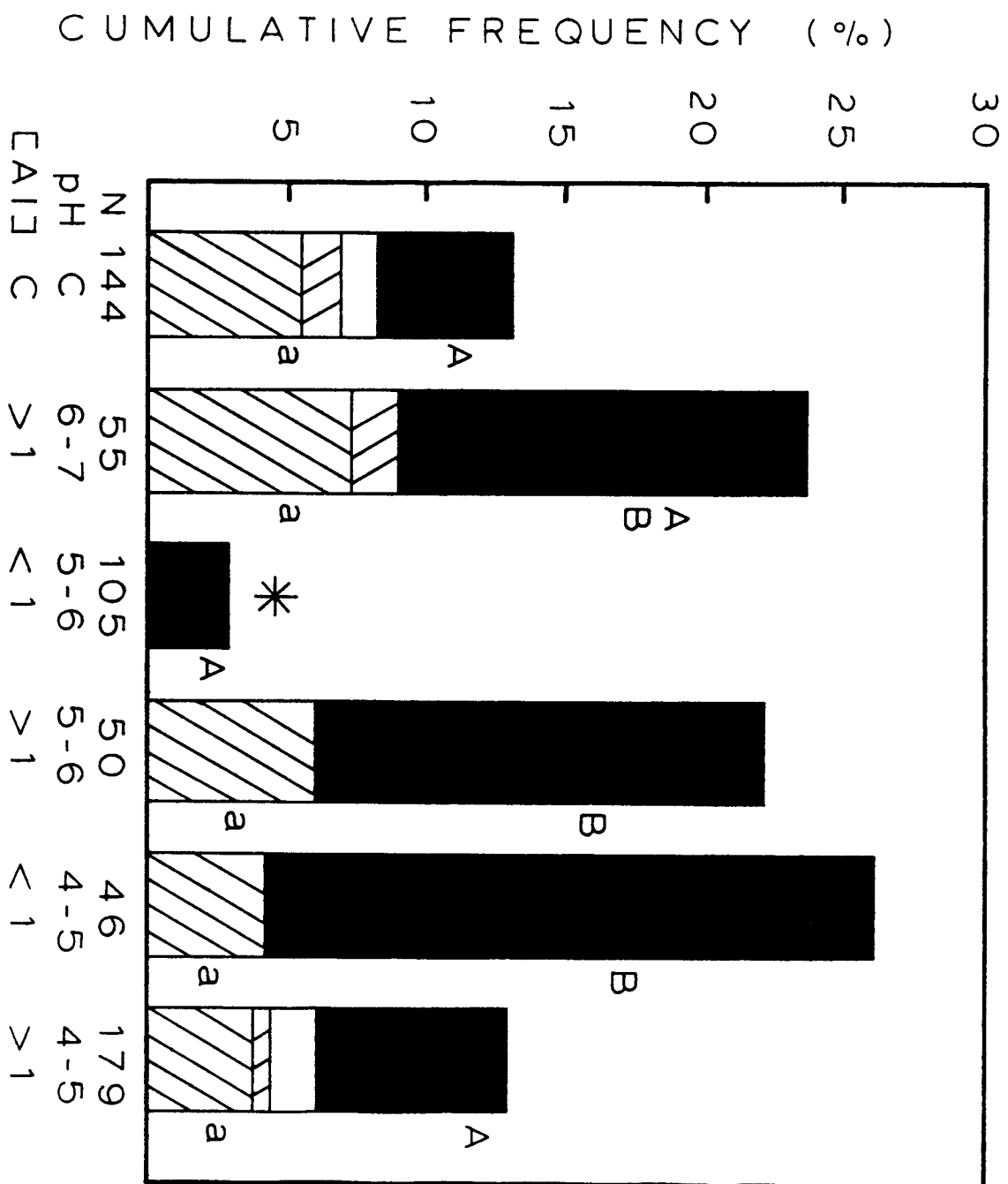
FIGURE 6

FIGURE 7

FIGURE 8

CUMULATIVE FREQUENCY (%)
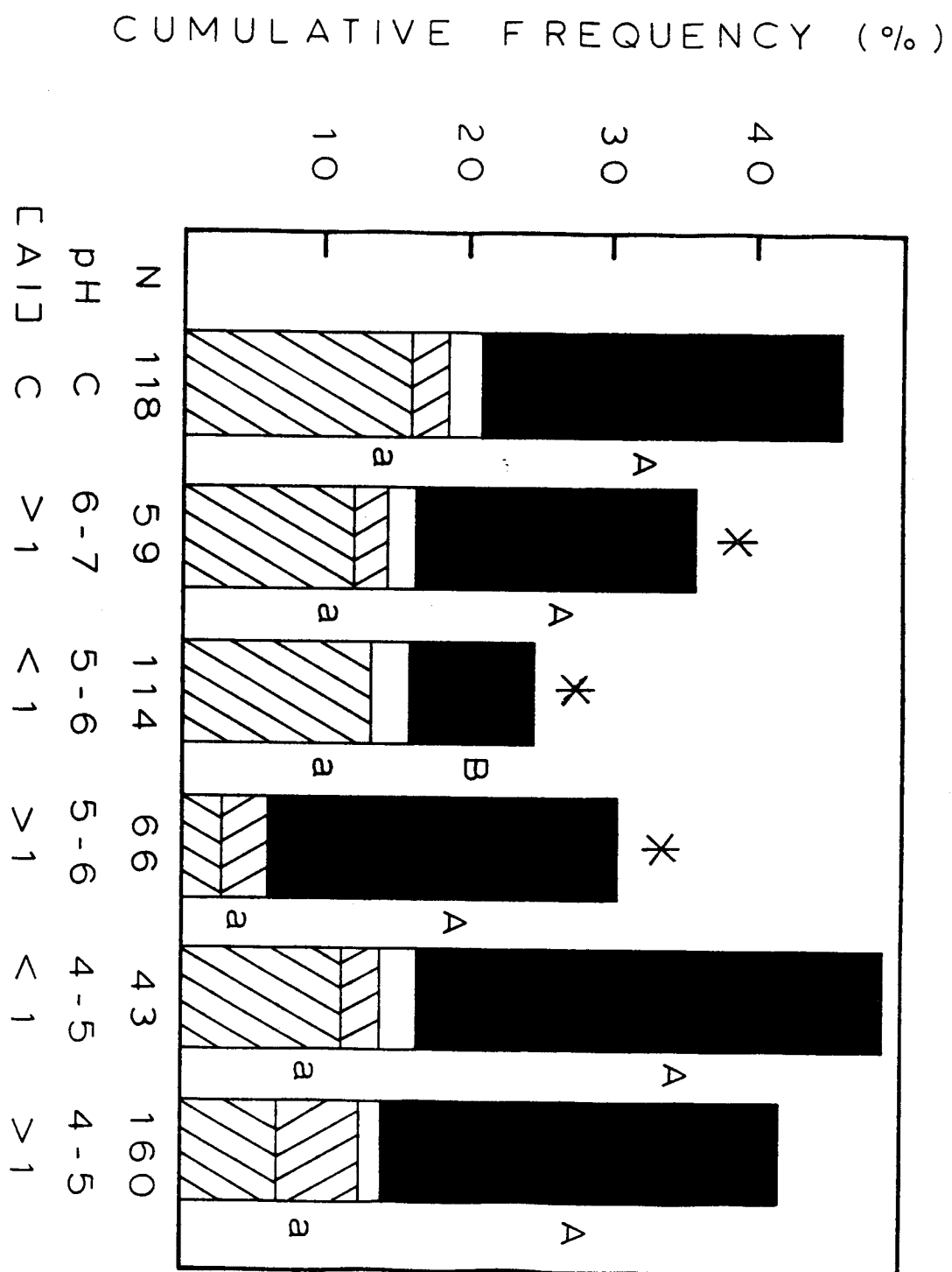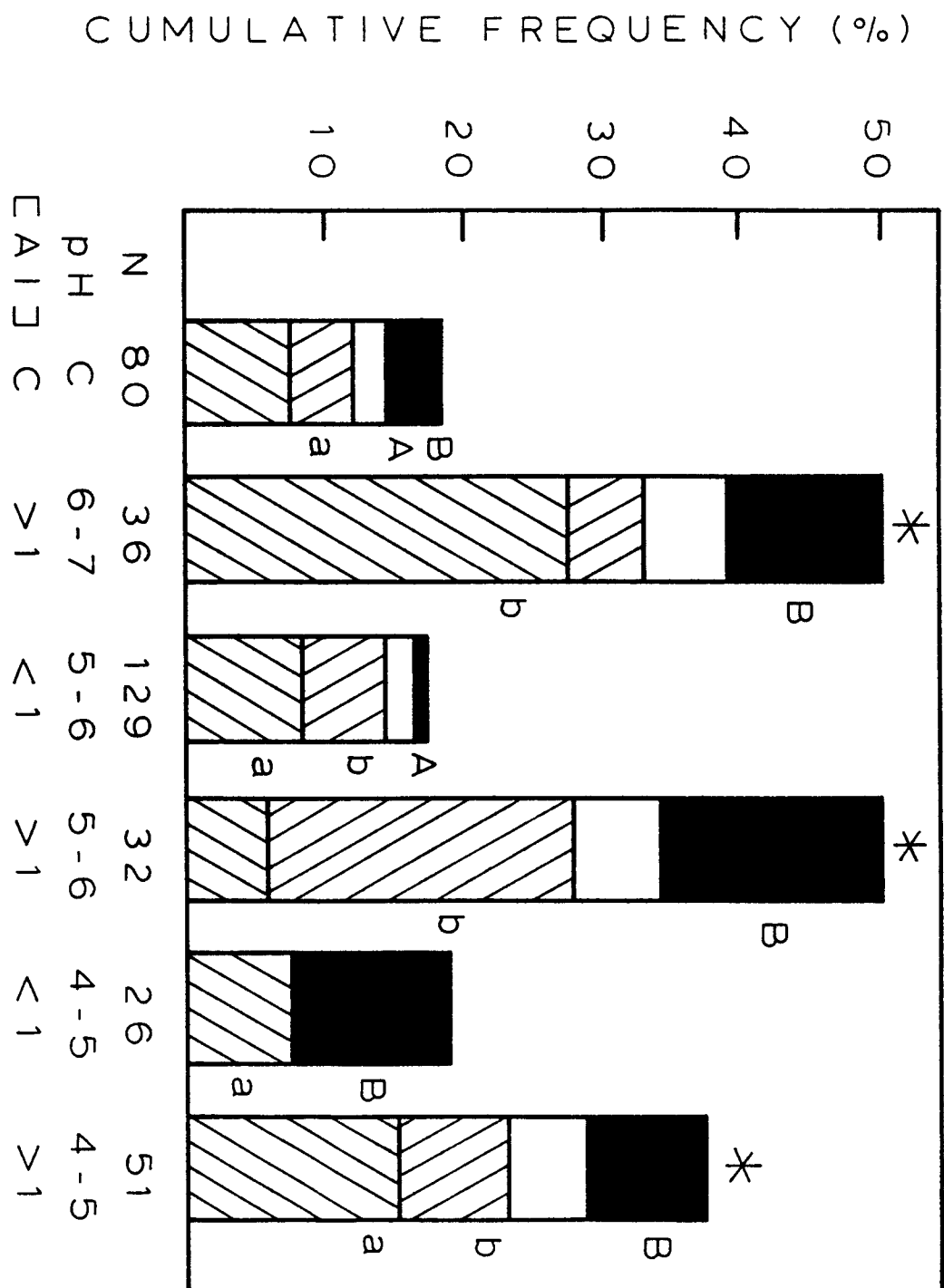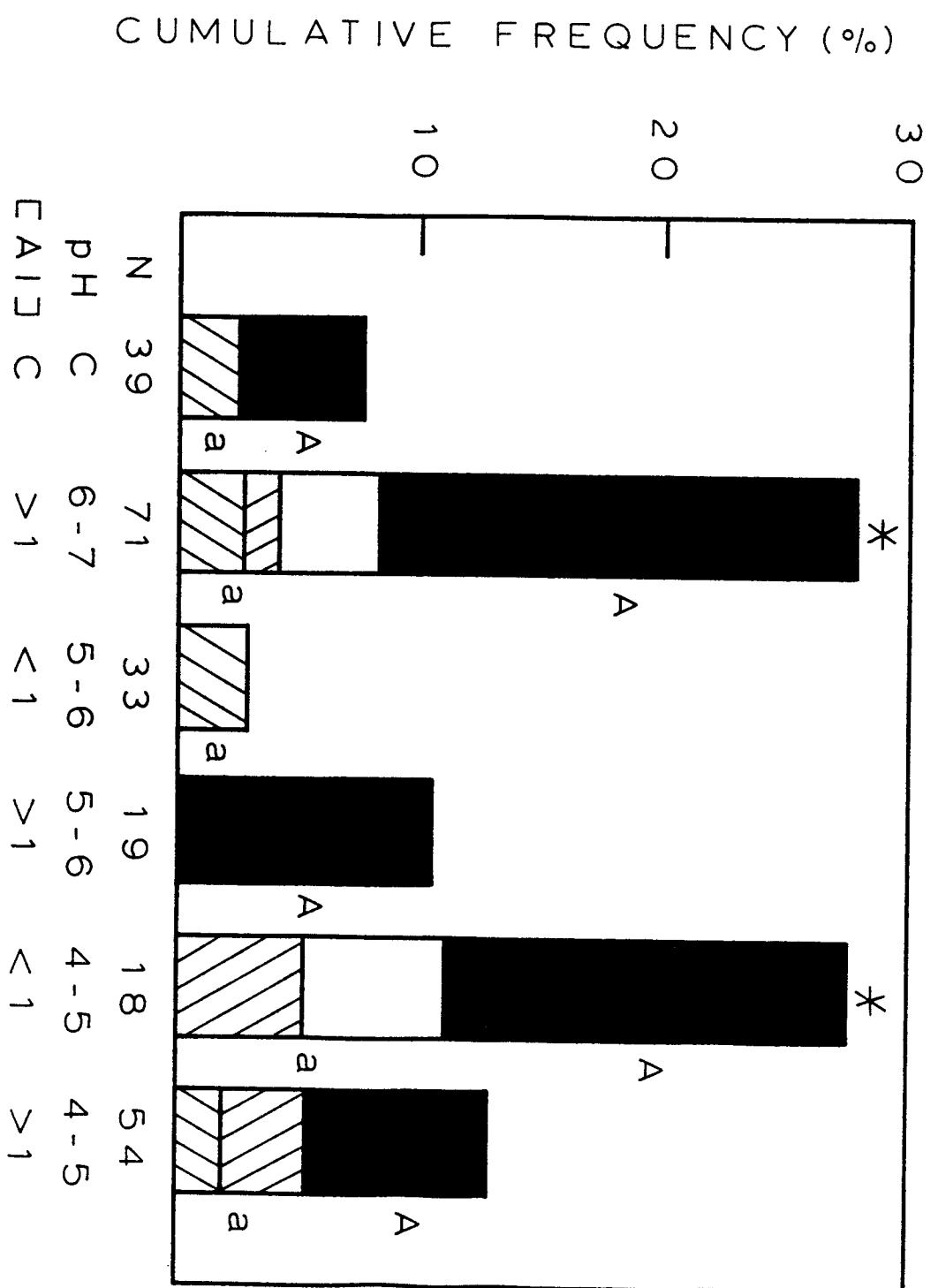
FIGURE 9

FIGURE 10

FIGURE 11

VCR

M

PI

pH

RAD

temp
O_2

1 2 3 4

STREAM

WINDOW

FIGURE 12

16 JULY 1985

ACTIVITY (cm ind⁻¹ min⁻¹)

$$ACTIVITY \ (cm \ ind^{-1} \ min^{-1})$$

TIME

FIGURE 13

23 JULY 1985
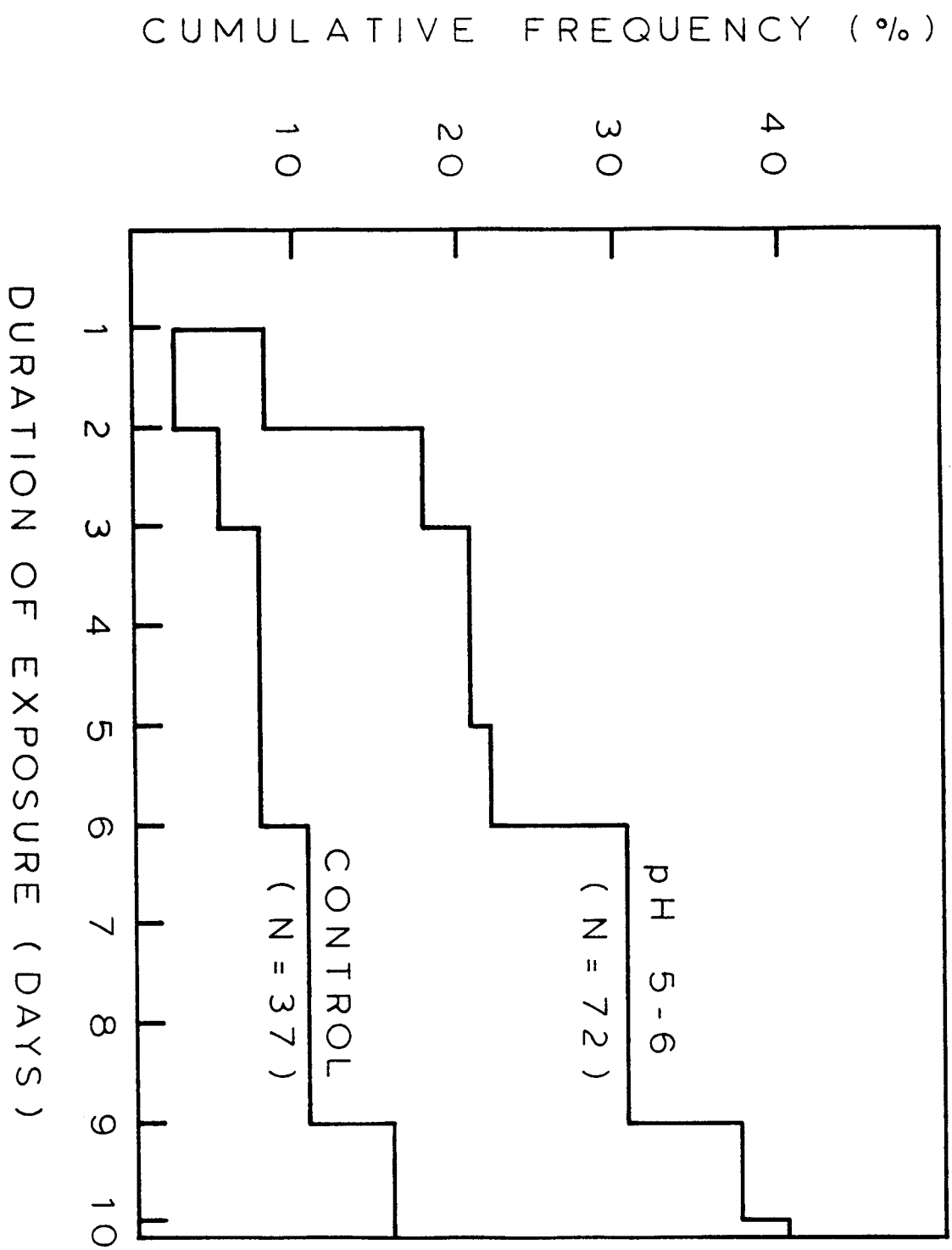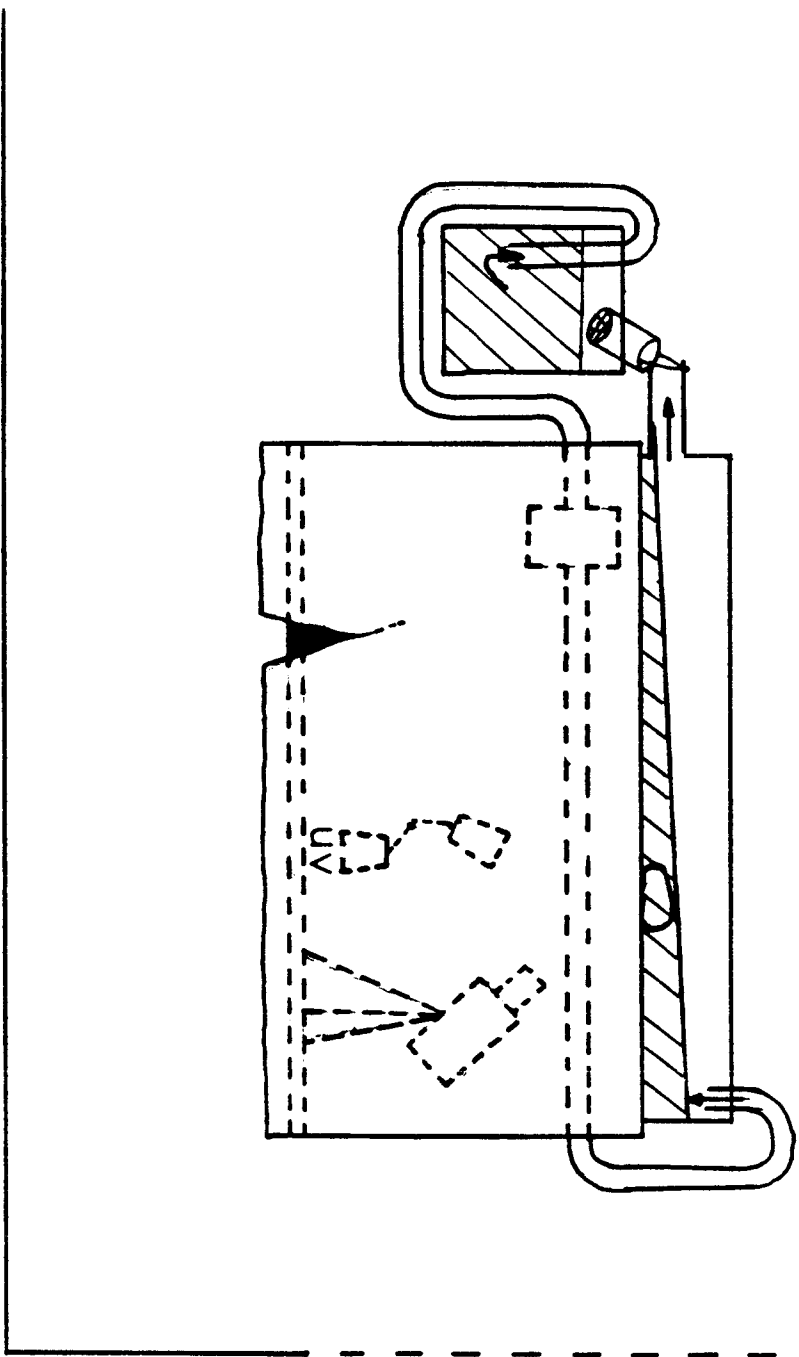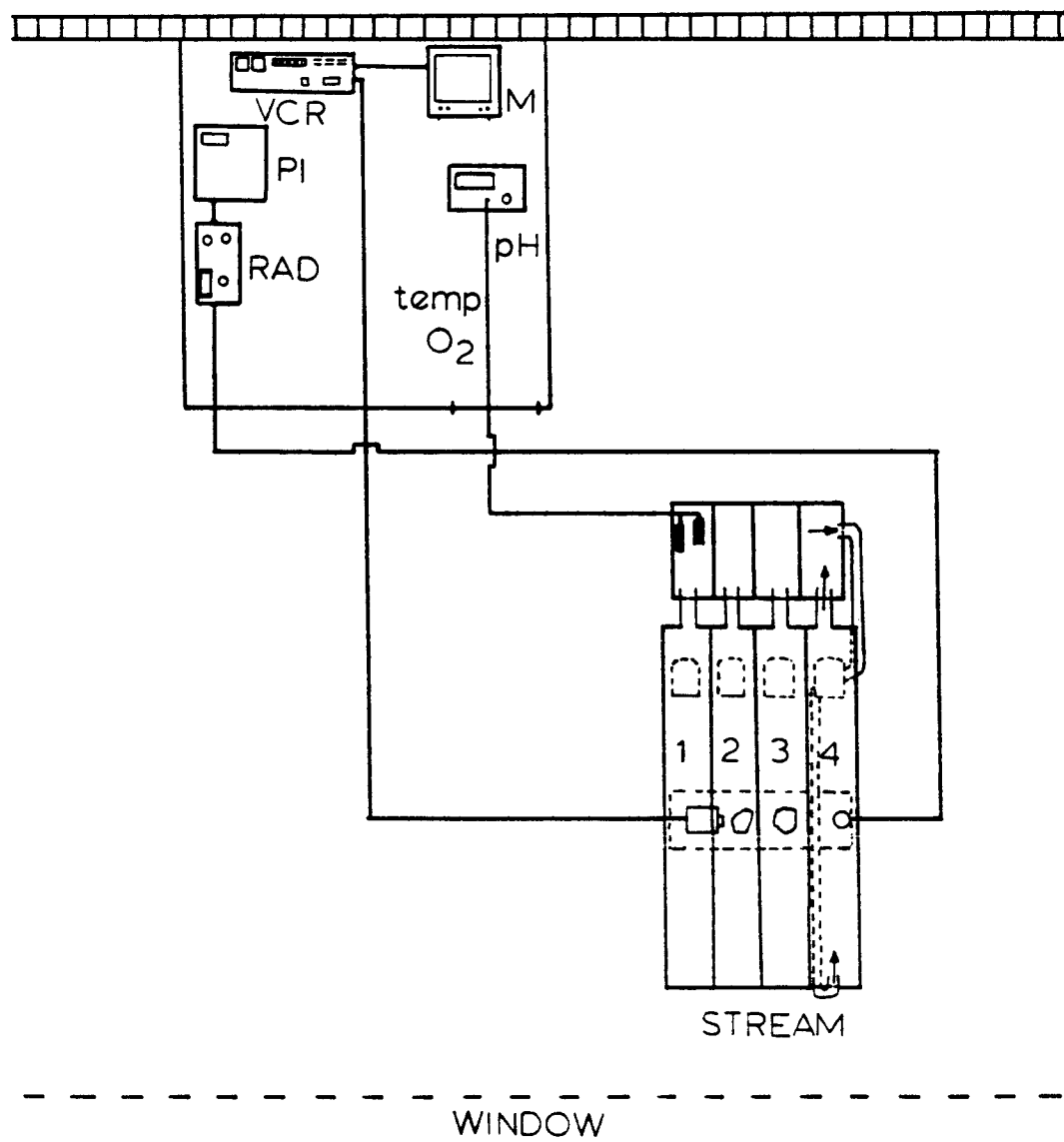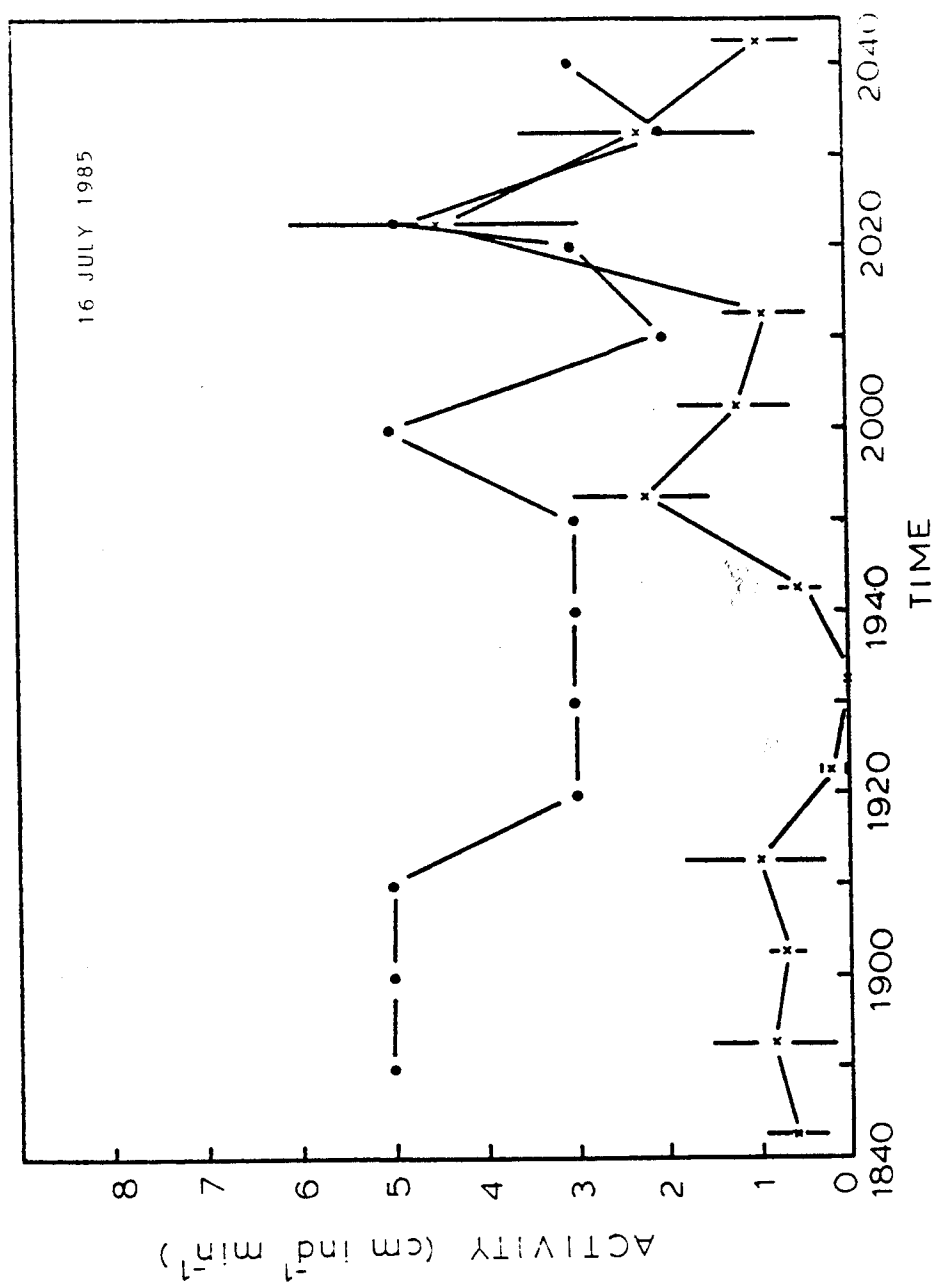
FIGURE 14

FIGURE 15

FIGURE 16